9

Changes in the Cytological Structure of the Adenohypophysis and Gonads in Juvenile *Bathygobius soporator* after Pituitary Implantation[1]

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(Plates I & II)

Introduction

THE relationship of the pituitary and the gonads has been studied and acknowledged in all vertebrate classes. General seasonal changes in the pituitary and gonads of the goldfish and carp were described by Scruggs (1951) and correlated with the previous literature on the subject in teleosts. No report has been found on the histology of the the specific cell types of the pituitary correlated with the histology of the gonads after experimentally induced changes in the two tissues. The present report is a histological study of gonads and transitional lobes of pituitaries of juvenile fish after pituitary implantation.

All fish used were collected in shallow water areas around North Bimini Island, Bahamas, B. W. I. Experimental work was done at the Lerner Marine Laboratory on Bimini.

The help given during this study by Dr. C. M. Breder, Jr., and Miss Priscilla Rasquin is gratefully acknowledged.

Materials and Methods

The marine teleost *Bathygobius soporator* (Cuvier & Valenciennes) was used. This species is very hardy and will survive the handling involved during implantation. Since these fish do not engage in active swimming to a large extent, the incision healed rapidly and no stitches were necessary.

Twenty-five young *Bathygobius soporator* of an average standard length of 38 mm. were given intraperitoneal implants of whole pituitary glands from larger fish of the same species. The average standard length of the donors was 66 mm. All 25 fish received three pituitaries each.

Pituitaries were obtained by the following procedure. The donor fish was heavily anesthetized in ethyl urethane dissolved in sea water. The lower jaw was removed and the floor of the cranium with its covering membranes was cut away with small bone clippers, exposing the pituitary. The area for cutting was determined by the position of the saccus vasculosus, which could be seen through the bone. Pituitaries were removed by grasping the infundibulum with watch-maker forceps and pulling gently. The pituitaries were held in physiological saline (0.6%) until all three were collected.

The recipient fish was lightly anesthetized with ethyl urethane and placed on cotton saturated in sea water. A slit was made in the abdominal wall lateral to the midline with watch-maker forceps and the three pituitaries were introduced through the slit. A dissecting microscope was used while removing and implanting the glands. Immediately after implantation, the fish were placed in individual two-gallon tanks with running sea water.

The gonads of the donor fish were removed and fixed in Bouin's picro-formol solution, embedded in paraffin, sectioned at 10μ, and stained with Harris's haematoxylin and eosin. The fish receiving the implants were killed after varying lengths of time. Eighteen fish were killed at seven to eight days, and seven fish from two and one-half to three days post-operative. Their bodies, minus the heads, were fixed in Bouin's, sectioned at 10μ through the region of the gonads and stained with Harris's haematoxylin and eosin. Heads of the 25 experimental fish were fixed,

[1]This study was supported in part by a grant from The National Science Foundation.

embedded in paraffin and sectioned at 5μ. The modification of Halmi's paraldehyde fuchsin stain by Sokol (1953) provided an excellent stain with which to observe the response of any specific cell group of the pituitary to a given experimental procedure. Bouin's fixative and the paraldehyde fuchsin stain were used for the heads of 18 of the experimental fish, and Baker's fixative and acid haematin stain were used for the seven other experimental fish. The paraldehyde fuchsin was used specifically for the study of basophils and the acid haematin for acidophils.

A group of 11 normal fish were killed and prepared for histological study by the same methods used for experimental fish. The normal fish ranged from 80 mm. to 29 mm. in standard length. All stages of normal gonad development and maturation were represented in this series.

Tavolga (1955) reported that *B. soporator* collected in the area of Marineland, Florida, were at the height of the spawning season in July and August. However, the largest gobies which were collected for the present study in Bimini waters were judged by gross dissection and histological section to be in or near spawning condition in March and April. Testes were swollen, nearly white in color and had high epithelium; ovaries were bright yellow and thin walled as described by Vivien (1941) as characteristic of the spawning condition for the European goby, *Gobius paganellus*.

The largest female collected by Tavolga (1954) was 65 mm. in standard length, while males of 90 mm. or more were found. In Bimini, the largest female found was 87 mm. and the largest male 108 mm. The mean, of course, was lower, the average of 43 females being 66 mm. and of 56 males, 74 mm.

Testes of eight normal *Bathygobius* were stained with Sudan Black B and by the Baker technique for phospholipids. The testes of one fish which had received three pituitary implants and maintained for one week was also stained with Sudan Black B.

Results

Implants of pituitaries of mature fish to juvenile fish caused degranulation of the basophils of the adenohypophysis and stimulation of the gonads. The acidophils of the transitional lobe were not affected. In both experimental and control fish, the acidophils were large, well-rounded and heavily stained with the Orange G of the paraldehyde fuchsin stain. The acidophils stained with acid haematin did not show degranulation.

Degranulation of the basophils occurred in nine fish which had received implants and were maintained for one week before being killed. Little or no degranulation was seen in seven fish sacrificed three days after receiving pituitary implants, nor in two fish maintained for one week before killing. The latter had gonads in very early stages of maturation and it is thought that the tissue may be refractory at this early stage. Partially degranulated basophils of a three-day implant are shown in Plate I, Figure 1, and may be contrasted with the advanced degranulated condition found in a seven-day implant shown in Figure 2. Normal acidophils are shown in Figure 3.

The degranulated basophils are found along the periphery of the pituitary and in groups along the region of juncture with the intermediate lobe. The centers of the cells are clear and the cells somewhat shrunken and collapsed. Dark-staining granular material may be seen between the cells. The basophils of the control and non-reactive experimental fish are large and have either very small clear areas or none. The basophils take a very heavy and dark stain.

Atz (1953) described basophils similarly located in the fresh water teleost, *Astyanax mexicanus*. The correlation of changes in the peripheral basophils with changes in the gonads led her to consider these cells as gonadotrophs. In all but one case basophils were reported to have increased in number near the time of onset of the spawning season. In no case was there a report of degranulation occurring at this time. In the present experiment no increase in the number of basophils was noted.

The possibility of basophilic degranulation occurring as a result of stress and loss of ACTH was considered. However, two fish which survived seven days showed no degranulation and the seven fish which were killed after three days, an even shorter time to adjust to stress, showed little or no degranulation.

The testes of *Bathygobius soporator* have an unusual amount of tissue which is not spermatogenic. The functional germ cell tissue of the testis is spirally wrapped around a core of tissue of very different nature. The core tissue has some resemblance to interstitial tissue but the amount seems out of proportion to the need or to the amount of interstitial tissue found in other teleosts. The intimate relation of the germ cell tissue and the core tissue would indicate that the core is a functional part of the testis but no definite function has been described.

Gonads, especially the ovaries, were stimulated in the fish showing degranulation. The stimulation caused rapid maturation of about half the ova and a third of the spermatogonia but little increase in over-all size of the gonad.

However, the core tissue of the testes hypertrophied. Stimulation was greater when the pituitary donor had gonads in intermediate stages of maturation than when the gonads were very advanced and approaching or in spawning condition. An ovary from an untreated juvenile and an untreated mature fish are shown in Plate I, Figures 4 and 5. Figure 6 shows the response of an ovary to the experimental procedure. The ovary from the mature untreated fish shows more uniform graded development than that of the treated juvenile fish where several ova are highly developed while the rest are in a uniform early stage.

Testicular and core tissue of an untreated juvenile and untreated mature fish are shown in Plate II, Figures 1 and 2. The normal condition of the core tissue of a juvenile fish stained with Sudan Black B to show lipids is shown in Plate II, Figure 3, and is contrasted with Figure 4 of the same magnification which shows the hypertrophied core tissue from a fish which had received pituitary implants. Baker's acid haematin and pyridine extraction techniques were used on the core tissue to indicate some of the cellular components. Staining results of the core tissue of normal fish showed a positive reaction to acid haematin (Plate II, Figure 5) and a negative one to pyridine extraction (Plate II, Figure 6), indicating the presence of phospholipids.

Discussion

The histology of the pituitary and the identification of cell types as sites of specific hormone production is still to some extent controversial. In the teleosts the transitional lobe has been found to be homologous to the anterior lobe in other vertebrates (Charipper, 1937).

The pituitary basophils of the rat were described as the site of thyrotropic and gonadotropic hormone elaboration and storage by Purves & Griesbach (1951). A subsequent report (1954) designated the specific basophils which produce TSH, FSH and LH.

However, there is some disagreement as to whether the gonadotropic hormone is a single biochemical entity or two or even more. Evans & Simpson (1950), in a review of the literature concerning the gonadotropins, called attention to the fact that it is not certain that there are two distinct gonadotropic hormones identical with the two substances which have been isolated from pituitary tissue or that both hormones are necessary in both sexes for normal reproductive function. The gonad maturity at the time of pituitary injection for experimental purposes is an important factor.

Matthews (1939), using injections of mammalian pituitary extract on *Fundulus heteroclitus,* found that the injections had no stimulating effect in the males and only four of 35 females responded to any degree. Hypophysectomy caused regression of the gonads especially in the males. On the basis of this work the pituitary gland of a teleost was seen to exert a controlling influence on the seasonal cycle and this influence was considered to be "... of greater importance in maturation than in proliferation of the germ cells." The present study is in agreement, as pituitary implantation caused an acceleration in the rate of maturation of the germ cells.

The effect of adult *Fundulus* pituitary implants to immature fish of the same species was reported by Matthews in 1940. Adult pituitary implanted intraperitoneally into immature fish at three-day intervals caused gonad stimulation by the end of four weeks. The males were especially responsive and large numbers of mature spermatozoa were seen. The pigmentation of the fish was characteristic of the adult in breeding season. Included in this report was a review of the use of mammalian pituitary extracts for injection in fish and the conflicting results and data collected; in many cases the mammalian preparation had no effect. A similar review of the use of injecting fish pituitary preparations in fish showed a fairly uniform response. All species tried showed enlargement of the gonads and in some cases expulsion of eggs and sperm. All experiments involving hypophysectomy showed gonad regression.

Burger (1941) used pituitaries from adult *Fundulus* as implants to other adult male *Fundulus* which had been hypophysectomized at the time of maximal testicular development and which showed inhibition of the testes after hypophysectomy. The implants caused recrudescence of the testes within two weeks. The implants were made intraperitoneally and each fish received five at a time for four times. Burger concluded that the *Fundulus* pituitary contained gonadotropic material which was responsible for spermatogonial proliferation and for the maturation phenomena.

Riley & Fraps (1942) investigated the gonad-stimulating activity of anterior pituitary in the female domestic fowl. Glands from hens with regressed or quiescent ovaries produced a greater stimulation of the gonads in immature mice than glands from hens in full reproductive condition. Greater gonad stimulation of the gobies in the present report resulted when pituitary donors were in intermediate stages of gonadal development.

The cyclic changes in the pituitary of the urodele amphibian *(Taricha torosa)* were described by Miller & Robbins (1955). In this form the

delta basophils of the pituitary increase in number and granulation in relation to spring spermatogenesis and oogenesis and late fall final gonad maturation. The beta basophils were considered to be related to increased thyroid activity.

As part of a study of light and temperature effect on the sexual cycle of the bitterling *Rhodeus amarus,* Verhoeven & van Oordt (1955) studied the adenohypophysis of the fishes which had an experimentally induced sexual cycle. They briefly note that the beta cells of the gonadotropic zone had become more numerous and staining response of these cells to PAS was a deep purple coloration which in rats indicates gonadotropic hormone. The method by which an increase in the number of beta cells was ascertained was not given.

Rasquin & Stoll (1955) described the association of degranulated centrally located basophils with hypertrophied adrenals in the freshwater teleost *Astyanax mexicanus* after injections of pitressin. The degranulation was thought to be a result of loss of ACTH, as the thyroid tissue was not stimulated. The peripheral basophils (suspected of gonadotropin elaboration) did not degranulate.

The only possible explanation for the appearance of degranulated basophils after pituitary implantation in the present study is that the basophils are not elaborating secretion granules at the normal rate because of the excess pituitary substance which was added. The absence of granules in cells usually crowded with them is generally interpreted as a release of hormone, but since the fish were given more pituitary there would appear to be no need for the pituitary of the implanted animal to add its secretion to a system already over-supplied. The fact that the degranulation was so gradual—little or none could be detected after three days of implantation—supports the idea that degranulation in this case is a result of an inhibition of elaboration rather than a stimulated release of secretory products which would presumably occur more rapidly.

Sections of the core tissue of testes of *Gobius auratus* Risso were stained and found to be faintly fuchsinophilic and unblackened by iron haematoxylin (Eggert, 1931). The granules within the cells of the tissue apparently contained lipids. Included in the report is a survey of the literature on the core tissue and a discussion of the possibility that this is the interstitial tissue. Eggert claimed that the fine vacuolation and granulation indicated that the cells were not interrenal. He did not find core tissue in the testes of *G. panizzae* Verga, *G. buchichi* Steindachner, *G. quagga* Heck or *G. jozo* Linnaeus.

Coujard (1941) described the core tissue in *Gobius niger* and *G. minutus* as being made up of cells with large nuclei and nucleoli, abundant in protein and containing lipids. He did not attribute a definite function to the tissue but considered that it undoubtedly had a function correlated with glandular development and the nervous mechanism of spawning.

The procedure for demonstrating phospholipids has been described in detail by Baker (1946). He used the two-stage technique of acid haematin staining and pyridine extraction on several tissues, including the testes of the mouse. The secretion droplets of the interstitial cells reacted positively to acid haematin and negatively to pyridine extraction, which identified the secretory granules as phospholipid.

Melampy & Cavozos (1954) made a comparative study of lipids in the vertebrate testis, using Sudan dyes and Baker's acid haematin-pyridine extraction technique. Using the Sudan dyes, a positive reaction was obtained from the interstitial cell cytoplasm, basement membrane, cytoplasm of Sertoli cells, spermatogonia, spermatocytes and spermatids of the bull, ram, boar, guinea pig, rooster and horned lizard. The teleost, the bluegill *(Lepomis macrochirus),* showed a negative reaction with the Sudan dyes. The authors suggest that the failure to get a positive reaction from bluegill testis might be due to the cyclic breeding season of this form and that at the stage of the test the reactive quantity of cholesterol in the testis was absent. Baker's test was not applied to the bluegill testis.

The implantation experiments reported here show that the core tissue of the testis is responsive to pituitary stimulation. The marked hypertrophy of the cells, the high phospholipid content and the close proximity of the core tissue to the spermatogenic cells seem sufficient evidence for designating the core cells as interstitial tissue.

Summary

1. Pituitary implants from adult fish to juvenile fish caused degranulation of the peripheral basophils of the transitional lobe of the hypophysis and gonad stimulation in receiver fish maintained for one week after implantation.

2. Basophils of fish maintained for three days after implantation showed little or no degranulation.

3. Acidophils of the adenohypophysis were not affected by the experimental procedure.

4. Degranulation which occurred gradually may be the result of inhibition of hormone elaboration rather than stimulation of release of hormone.

5. Basophilic degranulation, when it occurred,

was the same in both sexes but acceleration of maturation of the ovary was greater than that in the testis.

6. The core tissue of the testes hypertrophied after pituitary implantation and had a high phospholipid content. On this evidence, the core tissue is designated as interstitial tissue.

7. The degree of gonad maturity of the pituitary donors was an important factor in the results. Greater stimulation resulted when donors were in intermediate maturation stages than when donors were in advanced stages.

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EXPLANATION OF THE PLATES

PLATE I

FIG. 1. Partially degranulated basophils in a 41 mm. fish three days after pituitary implantation. Paraldehyde fuchsin, sagittal section, 1500×.

FIG. 2. Peripherally located degranulated basophils from a 40 mm. fish killed one week after pituitary implantation. Paraldehyde fuchsin, sagittal section, 1500×.

FIG. 3. Normal acidophils and basophils from 43 mm. control fish, paraldehyde fuchsin, sagittal section, 1500×.

FIG. 4. Ovary of 55 mm. untreated juvenile. Haematoxylin and eosin, cross section, 100×.

FIG. 5. Ovary of 73 mm. untreated adult. Haematoxylin and eosin, cross section, 100×.

FIG. 6. Stimulated ovary from 42 mm. fish after one week of pituitary implantation. Haematoxylin and eosin, cross section, 100×.

PLATE II

FIG. 1. Core and spermatogenic tissue of 41 mm. untreated juvenile. Haematoxylin and eosin, cross section, 100×.

FIG. 2. Core and spermatogenic tissue of 80 mm. untreated adult. Haematoxylin and eosin, cross section, 100×.

FIG. 3. Core tissue of 41 mm. untreated juvenile stained with Sudan Black B, cross section, 500×.

FIG. 4. Core tissue of 35 mm. juvenile after one week of implantation. Sudan Black B, cross section, 500×.

FIG. 5. Core tissue of 81 mm. adult stained with acid haematin, cross section, 1500×.

FIG. 6. Core tissue of 74 mm. adult after pyridine extraction, cross section, 1500×.